IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#23

In re Application of	
Junichi Shimada, et al.	
Serial No. 09/486,823	Group Art Unit: 1614
Filed: March 03, 2000	Examiner: Phyllis G. Spivack
For: Therapeutic Agent for Neurodegenerative Disorders)	

DECLARATION

The Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Shunji Ichikawa of 825, Kannami-cho Hita, Tagata-gun, Shizuoka, 419-0125, Japan, do declare as follows:

I joined Kyowa Hakko Kogyo Co., Ltd in April, 1969. During 1971-1973, I was engaged in the research on the efficacy and general pharmacology of L-DOPA as an antiparkinson drug in rodents. During 1974-1977, I was engaged in the examination of the anxiolytic and general pharmacological effects of flurazepam in rodents. I was involved in the general pharmacological tests for micronomicin sulfate, an aminoglycoside antibiotic, and levamisole hydrochloride in rodents during 1983-1986 and 1987-1990. From 1990, I was engaged in the evaluation of the facilitatory effects of domperidone etc. on gastrointestinal motility in animals. Since 2000, I have been in charge of efficacy pharmacology and safety pharmacology in the exploratory pharmacology department

at Pharmaceutical Research Institute of the company.

The following experiment was conducted under my direction.

EXPERIMENT

The protective effects of **Compound 1** of the present application and **Compound 10** disclosed in Table 3 of Baraldi et al., Current Medicinal Chemistry, against cerebral ischemia in Mongolian gerbils were examined.

Method:

Male Mongolian gerbils, 10 weeks old, were used. Ten animals were assigned to each group. Reserpine (5 mg/kg) was injected intraperitoneally to the gerbils. One day later, the right carotid artery of each gerbil was completely occluded under anesthesia. At 30 minutes after the occlusion, the animals were given vehicle (0.5 % methyl cellulose), 0.1 mg/kg of **Compound 1** or **Compound 10** orally and then monitored with respect to the mortality for 12 hours after the occlusion. The result is shown in Table 1.

Result:

Table 1. The protective effects against cerebral ischemia in Mongolian gerbils

Drugs (0.1 mg/kg, p.o.)	Survival animals / Total animals (at 12 hours after occlusion)
Vehicle	0 /10
Compound 1	10 /10
Compound 10	6 /10

Conclusion:

All animals died in the vehicle group within 12 hours after the occlusion. However, **Compound 1** and **Compound 10** prevented the mortality. Moreover, the protective action of

Compound 1 was more effective than C mp und 10.

The undersigned declarant declares further that all statements made herein of his knowledge are true and that all statements made on information and b lief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Executed this 15 day of July , 2003.

Shunji Ichikawa

Shunji ICHIKAWA

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DECLARATION

The Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Masako Kurokawa of 656-18, Yasuhisa, Mishima-shi, Shizuoka, 411-0815, Japan, do declare as follows:

I finished my bachelor course at Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kyushu University in March, 1988, and I was given the degree of B.A. In July 1997, I took the degree of Ph.D. at Faculty of Pharmaceutical Sciences, Kyushu University. Since April, 1988, I have been employed by Kyowa Hakko Kogyo Co., Ltd., the assignee of the above-identified application, and engaged in the research on neuroscience (screening of new drugs and investigation for

the mechanism of action of drugs) at Pharmaceutical Research Institute of the company. From June to August 1993, I was sent to Department of Pharmacology, University of Cambridge in U.K. for the study on the basic research in a neuroscience field.

The following experiment was conducted under my direction.

EXPERIMENT

The neuroprotective activity of **Compound 1** of the present application on MPTP-induced dopaminergic neurodegeneration was compared with that of **Compound 5** in EP 0698607.

Method:

The experiment was conducted according to the method of Test Example 1 in the specification of the present application.

In the experiment, male C57BL/6NCrj mice (11 weeks old, Nippon Charles River) were used. The mice were intraperitoneally treated with 1-methyl-4-pheny-1,2,3,6-tetrahydropyridine hydrochloride (MPTP HCl, 40 mg/kg, RBI Co., Ltd.). After 2 hours, vehicle (0.5% methyl cellulose), or each of **Compound 5** and **Compound 1** (1 mg/kg, in 0.5% methyl cellulose) was orally administrated. Seven days after MPTP HCl treatment, the mice were sacrificed by decapitation, and striatum was dissected out and stored at -80°C until [^{3}H]-mazindol assay. Each striatum was homogenized in microcentrifuge tube containing 300 µL of chilled buffer (120 mmol/L NaCl, 5 mmol/L KCl, 50 mmol/L Tris, pH 7.9), and centrifuged at 18000g for 5 minutes. The pellet was resuspended in 500 µL of the buffer. Assay mixture was consisted of 100 µL of tissue suspension, [^{3}H]-mazindol (10 nmol/L, NEN™ Life Science Products (Boston, MA, USA)), and incubated at 0°C for 1 h in the presence (non-specific binding) or absence (total

binding) of nomifensine (10 µmol/L). Then, the mixture was immediately filtrated through glass-fiber filters (Whatman, GF-B) and washed three times in 5 mL of the buffer. The radioactivity was measured by liquid scintillation spectrometry. Protein concentration of each sample was assayed using DC protein assay kit (Bio-Rad Laboratories) with bovine serum albumin as a standard. Control group was not treated. Specific [^{3}H]-mazindol binding was expressed as the amount of bound [^{3}H]-mazindol per unit weight of protein, mean ± standard error was determined for each group. The result is shown in Table 1.

Results:

Table 1.

Group	[^{3}H]-mazindol binding fmol/mg protein
Control	591 ± 33
MPTP HCl + vehicle	360 ± 26
MPTP HCl + **Compound 5**	381 ± 18
MPTP HCl + **Compound 1**	501 ± 53

Conclusion:

MPTP decreased [^{3}H]-mazindol binding in striatum to about 60% of control. In this model, **Compound 1** prevented the MPTP-induced reduction of [^{3}H]-mazindol binding and was more effective than **Compound 5**.

The undersigned declarant declares further that all statements made herein of her knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section

1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Executed this 15th day of July , 2003.

Masako Kurokawa

Masako Kurokawa